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Hemodynamics are altered in the caudal artery of beef heifers fed different ergot alkaloid concentrations[1]

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ABSTRACT: Doppler ultrasonography was used to compare blood flow characteristics in the caudal artery of heifers fed diets with endophyte (*Neotyphodium coenophialum*) noninfected (E−, 0 μg of ergovaline/g of DM), a 1:1 mixture of endophyte-infected and E− (E+E−; 0.39 μg of ergovaline/g of DM), or endophyte-infected (E+, 0.79 μg of ergovaline/g of DM) tall fescue (*Lolium arundinaceum*) seed. Eighteen crossbred (Angus × Brangus) heifers [345 ± 19 kg (SD)] were assigned to individual pens and fed chopped alfalfa hay plus a concentrate that contained E− tall fescue seed for 7 d during an adjustment period. A 9-d experimental period followed with feeding treatments of chopped alfalfa hay plus a concentrate with E+, E−, or E+E− seed being assigned randomly to pens. Doppler ultrasound measurements (caudal artery luminal area, peak systolic velocity, end diastolic velocity, mean velocity, heart rate, and flow rate) and serum prolactin were monitored during the adjustment (3 baseline measures) and during the experimental period (7 measures). Statistical analyses compared proportionate differences between baseline and responses at 3, 27, 51, 75, 171, and 195 h from initial feeding of the experimental diets. Serum prolactin concentrations for E+ and E+E− diets were less ($P < 0.001$) than baseline concentrations beginning at 27 and 51 h, respectively, from initial feeding of the diets. Although baseline measures were taken when ambient temperatures were likely below thermoneutrality, caudal artery luminal cross-sectional area in E+ heifers had declined ($P = 0.004$) from baseline by 27 h and remained less ($P < 0.02$) until 195 h, and caudal artery luminal area declined ($P = 0.004$) in E+E− heifers from baseline by 51 h and remained less ($P < 0.07$) until 171 h. Blood flow rate was slower than the baseline rate at 51 h for E+ ($P = 0.058$) and E+E− ($P = 0.02$ heifers, but blood flow remained slower in E+E− heifers for 48 h, whereas it remained slower in E+ heifers for 96 h. Adjustments in artery luminal area and blood rate with the 3 diets appeared to parallel the increases in ambient temperature. Heifers fed a diet containing a larger amount of ergot alkaloids had less of a response to ambient temperature than heifers consuming the diet with less or no ergot alkaloids.

Key words: beef cattle, Doppler ultrasonography, fescue toxicosis, *Lolium arundinaceum*, tall fescue, vasoconstriction

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INTRODUCTION

Tall fescue is a cool-season perennial grass that is widely utilized for grazing in the transition zone between the temperate northeast and the subtropical southeast (Thompson et al., 2001). Although tall fescue is well adapted to the climate and soils in the region, an endophytic fungus (*Neotyphodium coenophialum*) infects plants in greater than 90% of tall fescue pastures (Sleper and West, 1996) and produces ergot alkaloids that bind biogenic amide receptors to constrict peripheral vascular tissues and reduce the ability of the animal to dissipate body heat (Oliver, 1997, 2005). Constricted blood flow to peripheral tissues can lead to severe hyperthermia in warm ambient temperatures and hypothermic conditions in cold ambient temperatures. Prolonged cold ambient can lead to the "fescue foot" malady whereby signs range from lameness to necrosis of tissues of hoofs, tails, and ear tips (Strickland et al., 1993). Rhodes et al. (1991) reported that blood flow in steers fed high-endophyte diets is reduced by 50% to skin over the ribs. Aiken et al. (2007) used Doppler ultrasonography to measure hemodynamics through

[1]Mention of trade names or commercial products in the article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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the caudal artery of heifers fed diets with endophyte-infected tall fescue seed and reported vasoconstriction at 4 h after feeding the toxic diet.

Klotz et al. (2007) reported an in vitro vasoconstriction response of the bovine saphenous vein to a minute concentration of ergovaline (1×10^{-8} M) and suggested that vasoconstriction could be mediated through bioaccumulation of alkaloids in the vasculature. However, threshold concentrations in vivo have eluded researchers largely due to limited availability of sensitive real time assessment tools. An experiment was conducted to evaluate and compare blood flow in the caudal arteries of heifers fed diets containing 0, 0.39, or 0.79 μg of ergovaline/g of DM. Ergovaline is a potent vasoconstrictor (Klotz et al., 2007) and is the most abundant ergopeptine produced by the fescue endophyte (Lyons et al., 1986).

MATERIALS AND METHODS

A feeding experiment was conducted at the University of Kentucky Animal Research Center in Versailles, KY. The experimental protocol was reviewed and accepted by the Institutional Animal Care and Use Committee at the University of Kentucky. Weather data were collected from a weather station within 1 km of the experimental site.

Eighteen crossbred (Angus × Brangus) heifers (12 to 14 mo) were used in the experiment. Diets of the heifers did not contain ergot alkaloids from birth until the beginning of the experiment. The heifers were on bermudagrass [*Cynodon dactylon* (L.) Pers.] pasture before weaning in October of 2006 at the USDA-ARS Dale Bumpers Small Farms Research Center in Booneville, AR. The calves were fed bermudagrass hay and concentrate free choice until late November when they were transported to the Kentucky location. The heifers were placed in pens and fed a corn silage-concentrate ration free-choice until the beginning of the experiment.

The feeding trial was conducted from March 26 to April 17, 2007. Heifers were assigned randomly to 18 small pens (1.8 × 2.3 m) with concrete floors and under cover in a 3-sided barn. Three concentrate diets that contained endophyte (*Neotyphodium coenophialum*) noninfected (**E−**, 0 μg of ergovaline/g of DM), a 1:1 mixture of endophyte-infected and E− (**E+E−**, 0.39 μg of ergovaline/g of DM), or endophyte-infected (**E+**, 0.79 μg of ergovaline/g of DM) tall fescue (*Lolium arundinaceum*) seed were assigned to small pens in a completely randomized design with 6 replications. Heifers were assigned to treatments such that mean BW [345 ± 19 kg (SD)], and variance in BW was similar among diet treatments. Composition of the concentrate was ground soybean hulls (47.6% of DM), ground tall fescue seed (47.6% of DM), mineral premix (1.2% of DM), and molasses (3.6% of DM). Concentrates were formulated to target ergovaline concentrations of 0.39 and 0.79 μg of ergovaline/g of DM for the E+E− and E+ diets, respectively.

During an adjustment period, chopped alfalfa hay was fed to heifers in each pen for 7 d, followed by feeding of chopped alfalfa and concentrate with E− seed for 7 d. A 9-d experimental period followed with feeding the treatment diets (chopped alfalfa and concentrate with E−, E+E−, or E+ tall fescue seed). Alfalfa and concentrates were fed for all treatment diets to provide 54.4 and 45.6% of DM, respectively. Heifers had ad libitum access to alfalfa-concentrate mixtures during the adjustment and experimental periods at 0900 h. The mixture was fed at 0900 h each morning in quantities targeted to provide approximately 5% orts. Orts were collected daily and weighed to estimate daily DM consumption.

After the mixing of the concentrates, each concentrate was subsampled. Samples for each treatment diet were composited, freeze-dried (Botanique model 18DX485A freeze drier; Botanique Preservation Co., Peoria, AZ), ground through a 1-mm screen, and assayed for ergovaline and ergovalinine by HPLC florescence using a modification of a procedure developed by Yates and Powell (1988). Separation was conducted with an Alltima C18 150 mm × 4.6-mm column with 3-μm particle size (Grace Davison Discovery Science, Deerfield, IL). Elution solutions were 75 mM ammonium acetate (**A**) in water:acetonitrile (3:1, vol/vol) and acetonitrile (**B**). Elution gradient was 95:5 (A:B) 1 min; linear change to 60:40 (A:B) during next 15 min and maintained for 5 min; changed to 0:100 (A:B) in 1.5 min and maintained for 5 min; changed to 100:0 (A:B) in 1 min and maintained for 6 min before returning to 95:5.

Baseline ultrasound measures were taken during the adjustment period when the E− diet was fed on d 4, 5, and 7. All scanning sessions began at 1200 h and ended at approximately 1400 h. Ultrasound measures were taken during the experimental period at 3, 27, 51, 75, 171, and 195 h relative to initial feeding of experimental diets. Ultrasound scans of the caudal artery at the 4th coccygeal (Cd4) vertebrae were measured using an Aloka 3500 Ultrasound Unit (Aloka Inc., Wallingford, CT) with a UST-5542 (13 MHz) linear array transducer set to a 2-cm depth. The scanning protocol followed those described by Aiken et al. (2007), with the exception that 5 cross-sectional Doppler flow scans and 5 Doppler spectra were measured. Peak systolic velocity, end diastolic velocity, mean velocity, pulsatility index, and flow rate per minute (artery luminal area × mean velocity × heart rate) were measured over 3 cardiac cycles within each scan and then averaged over the 5 scans. Pulsatility index is often used to measure vascular resistance because of its positive relationship with resistance (Petersen et al., 1997). Ultrasound measurements were performed by a trained technician that had no knowledge of treatments imposed on individual heifers.

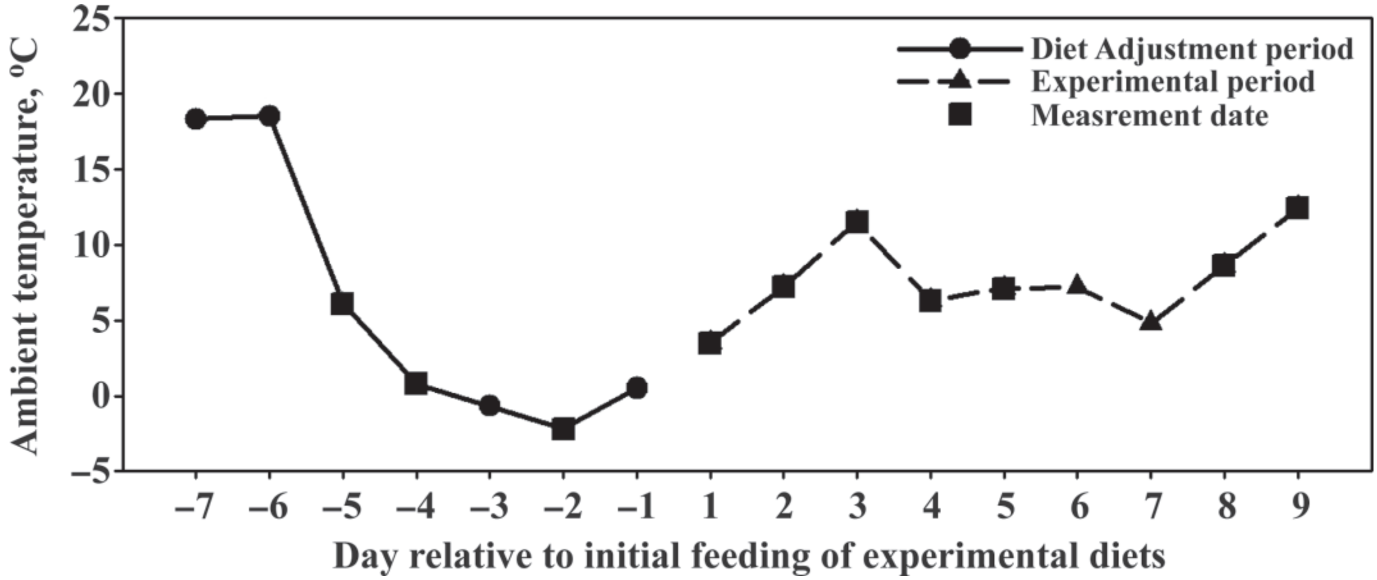

Figure 1. Average ambient temperatures recorded for each day during the adjustment and experimental periods. Day 1 was the first day the experimental diets were fed.

Approximately 10 mL of blood was collected from a jugular vein from each heifer during the adjustment period on d 4 and 5, and during the experimental period at 3, 27, 51, 75, 171, and 195 h relative to initial feeding of experimental diets. Blood was centrifuged for 15 min at 10,000 × g at 20°C to obtain serum, which was stored frozen (0°C). Serum was assayed for prolactin following procedures of Bernard et al. (1993). The intraassay CV was 7.86%.

The Shapiro-Wilk test for normality (Schlotzhauer and Littell, 1997) indicated that data for E− heifers were normally distributed for all variables except flow rate ($P < 0.01$). No data were normally distributed for any of the variables for the E+ ($P < 0.001$) or E+E− ($P < 0.10$) heifers. Therefore, the proportionate difference between response measures and baseline averages (response $_{\text{experimental period}}$ − baseline $_{\text{adjustment period}}$)/baseline $_{\text{adjustment period}}$) were statistically analyzed using the paired t-test (SAS Inst. Inc., Cary, NC) for each treatment diet at each hour from the initial feeding of E+ tall fescue seed. The analysis used baseline measures for individual heifers to calculate proportionate difference for replicates.

RESULTS

Temperature and Heat Indices

Mean daily ambient temperatures during the adjustment period averaged 5.9 ± 8.9°C and 7.6 ± 2.9°C during the experimental period, which likely resulted in the heifers being below their thermoneutral zone for much of the experiment (Figure 1). Ambient temperatures averaged 1.6 ± 4.2°C on days that animal data were collected during the adjustment period and averaged 8.1 ± 3.1°C on days that data were collected during the experimental period.

Toxin Concentrations

Chopped alfalfa hay plus concentrate containing E+ seed had ergovaline and ergovalinine concentrations of 0.79 and 0.38 μg/g of DM, respectively; the mixture with E+E− seed had ergovaline and ergovalinine concentrations of 0.39 and 0.13 μg/g of DM, respectively; and the mixture with E− seed had no detectable ergovaline.

Diet Consumption

Daily DMI averaged 7.9 ± 0.8 kg per heifer during the adjustment period when the E− diet was fed to all heifers, which was approximately 2.3% of BW. There was no treatment × date interaction ($P = 0.983$) on DMI during the experimental period, but consumption differed ($P = 0.069$) among treatments. Dry matter intake of the E+ diet (7.1 ± 0.4 kg) was less ($P = 0.025$) than of the E− diet (8.6 ± 0.4 kg), but DMI did not differ between the E+ and E+E− (8.1 ± 0.4 kg; $P = 0.118$) or E− and E+E− diets ($P = 0.416$).

Serum Prolactin

During the experimental period, serum prolactin concentrations averaged 191 ± 19 ng/mL for the E− diet, 102 ± 17 ng/mL for the E+ diet, and 142 ± 16 ng/mL for the E+E− diet (Figure 2). Baseline measures of serum prolactin of heifers fed the 3 diets averaged 254 ± 18 ng/mL and declined from baseline measures by 27 h with the E+ diet ($P = 0.012$) and by 51 h with the E+E− diet ($P < 0.001$; Figure 3). Prolactin concentrations with E+ and E+E− diets remained less ($P < 0.10$) than baseline concentrations for the remainder of the experimental period. Serum prolactin in E− heifers

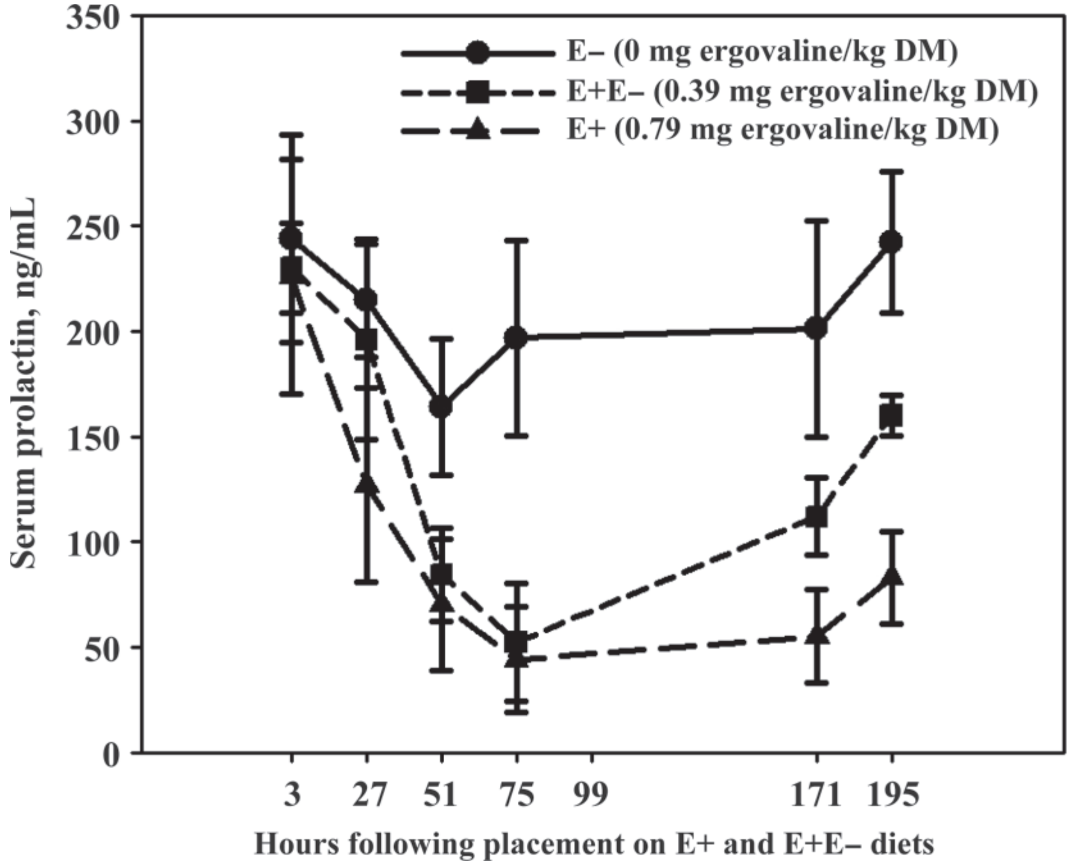

Figure 2. Serum prolactin concentrations measured during the experimental period for heifers fed diets with noninfected (E−; 0 μg of ergovaline/g of DM), a 1:1 mixture of E− and endophyte-infected (E+E−; 0.39 μg of ergovaline/g of DM), or endophyte-infected (E+; 0.79 μg of ergovaline/g of DM) tall fescue seed.

did not differ ($P > 0.18$) from baseline measures during the experimental period.

Doppler Ultrasound Measures

Cross-sectional area of caudal artery lumen during the experimental period averaged 3.4 ± 0.2 mm^2 for E+ heifers and 3.3 ± 0.3 mm^2 for E+E− heifers (Figure 4). The E− heifers averaged 5.4 ± 0.4 mm^2 during the experimental period but varied considerably between heifers (Figure 5a). Baseline caudal artery luminal area averaged 5.2 ± 0.2 mm^2. Artery area with the E+ diet was less ($P = 0.004$) than the baseline area by 27 h from the initial feeding of the diet (Figure 5c). Caudal artery luminal area for E+ heifers remained less than the baseline measure between 27 and 171 h from the initial feeding but increased to an area similar ($P = 0.548$) to the baseline at 195 h. For E+E− heifers, caudal artery luminal area declined to less ($P = 0.004$) than baseline by 51 h and remained less than baseline until the 99-h measurement ($P = 0.129$; Figure 5b).

Baseline heart rates for the treatment diets averaged 115 ± 2 beats/min. There were consistently negative proportionate changes in heart rate from the baseline rate for the E+ heifers, but significant reductions in heart rate were only detected ($P < 0.04$) at 27, 99, and 171 h from initial feeding of the diet (Figure 5f). Although the proportionate changes in heart rates from the baseline rate for the E+E− diet were consistently negative between 27 to 171 h from the initial feeding (Figure 5e), the reductions were not significant ($P > 0.13$). Heart rate with the E− diet was above the baseline at 3 h, but was similar to the baseline rate at each interval leading up to the final measurement at 195 h when it was less ($P = 0.037$) than baseline (Figure 5d).

Baseline measures for blood flow rates through the caudal artery for the 3 diets averaged 55.8 ± 7.7 mL/min. Blood flow rates for E+ and E+E− diets fell below baseline measures by 51 h ($P < 0.06$) from initial feeding of the experimental diets (Figure 5h, i). Blood flow rate for the E+E− diet had increased and was similar ($P = 0.73$) to the baseline flow by 99 h and remained similar to the baseline for the remainder of the experimental period. Blood flow for the E+ diet was less than the baseline ($P < 0.06$) from 51 to 171 h from the initial feeding, but was similar ($P = 0.546$) to the baseline at 195 h when the final measurement was taken.

There were no consistent changes in peak systolic, end diastolic, or mean velocities from baseline measures for each diet (Table 1). Each flow velocity with the E− diet did not deviate from baseline measures, with the exception of a greater mean velocity at 3 h from initial feeding of the experimental diets. Peak systolic velocity

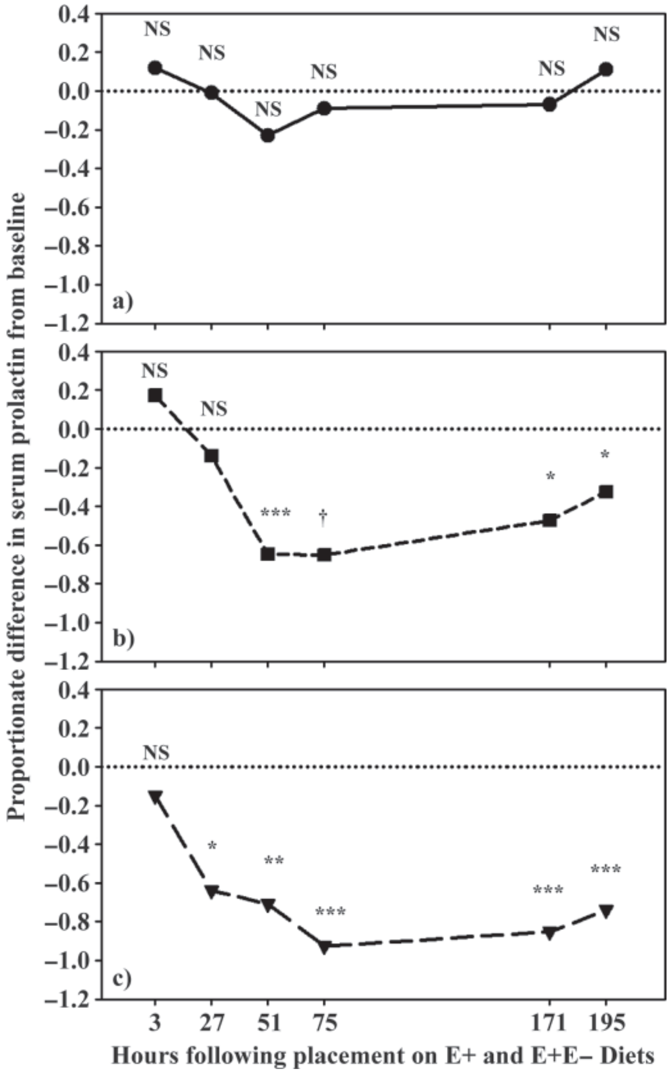

Figure 3. Proportionate differences between serum prolactin response to experimental diets and baseline measures. Baselines (·) were measured for individual heifers during the adjustment period. Treatment diets were (a) noninfected (E−; 0 µg of ergovaline/g of DM), (b) a 1:1 mixture of endophyte-infected and E− (E+E−; 0.39 µg of ergovaline/g of DM), or (c) endophyte-infected (E+; 0.79 µg of ergovaline/g of DM) tall fescue seed. Statistical differences from baseline measures denoted by $^*P < 0.10$; $^{**}P < 0.05$; $^{***}P < 0.001$; and NS = not significant ($P > 0.10$).

for the E+ diet was greater ($P = 0.094$) at 27 h but less than the baseline velocity at 75 ($P = 0.014$) and 99 ($P = 0.011$) h from the initial feeding. End diastolic velocity for the E+ diet did not vary ($P > 0.14$) from the baseline measure during the experimental period, and a deviation of mean velocity from the baseline was only detected ($P = 0.072$) at 75 h. Deviations from baseline velocities with the E+E− diet also were infrequent and detected primarily in the latter part of the experimental period when the experimental diets were fed. For the E+E− diet, peak systolic velocities were greater than baseline at 99 ($P = 0.92$) and 171 ($P = 0.028$) h, end diastolic velocity was less ($P = 0.011$) than the baseline at 75 h, and mean velocities were greater ($P < 0.10$) than the baseline at 3 ($P = 0.095$), 51 ($P = 0.10$), 171 ($P = 0.053$), and 195 ($P = 0.096$) h from the initial feeding. Pulsatility indices with the E− diet were less than baseline at 27 ($P = 0.015$) and 195 ($P = 0.066$) h, and with the E+ diet they were less than baseline measures at 27 ($P = 0.021$), 51($P = 0.003$), and 195 ($P < 0.001$) h. Pulsatility indices with both diets were greater than baseline measures at 75 h when the numeric differences from baselines for artery area and blood flows were greatest. Pulsatility indices for the E+E− diet were less than baseline at 99 ($P = 0.052$), 171 ($P = 0.052$), and 195 ($P = 0.031$) h and did not indicate any significant resistance relative to the baseline measure.

DISCUSSION

Body temperature responses to ergot alkaloids are typically detected with ambient temperatures above or below thermoneutrality (Spiers et al., 2005). Core body temperature of cattle consuming E+ tall fescue has been elevated compared with those consuming E− tall fescue as ambient temperatures increased above 31°C (Aldrich et al., 1993; Al-Haidary et al., 2001). Fescue foot is a malady manifested by fescue toxicosis in combination with cold ambient temperatures (Garner and Cornell, 1978; Cornell et al., 1982; Bacon, 1995) and is associated with excessive vascular constriction to extremities that leads to lameness and tissue necrosis. A vasoconstriction response due to the cold temperatures likely was prevalent (Sessler et al., 1990; Iwase et al., 2002) during the adjustment period when baseline measures were taken. As such, the differences in hemodynamics from baseline may have been greater for treatments had the temperatures during the experimental period remained similar to those during the adjustment period. Nonetheless, greater constriction that was measured with the E+ and E+E− diets after ambient temperatures increased clearly indicated the potency that ergot alkaloids have in inducing vasoconstriction relative to constriction caused by low ambient temperatures when the baseline measures were collected.

Serum Prolactin

Serum prolactin is a good indicator of fescue toxicosis because it is consistently suppressed in cattle grazing toxic tall fescue (Hurley et al., 1981; Lipham et al., 1989; Paterson et al., 1995; Aiken et al., 1998). The decline in prolactin concentration for the E+ diet by 27 h from the initial feeding indicates dopaminergic activity of the alkaloids on the anterior pituitary gland (Oliver, 2005) can be rapid with alkaloid concentrations (≥0.79 µg of ergovaline/g of DM) fed in this diet. Decline in prolactin concentrations for the E+E− diet was 24 h later than with the E+ diet, which supports the suggestion of Klotz et al. (2007) that there is bioaccumulation of ergot alkaloids before a response is mediated.

In a similar experiment, Aiken et al. (2007) concluded that a significant decline in serum prolactin for heifers fed E− seed was caused by low ambient temperatures.

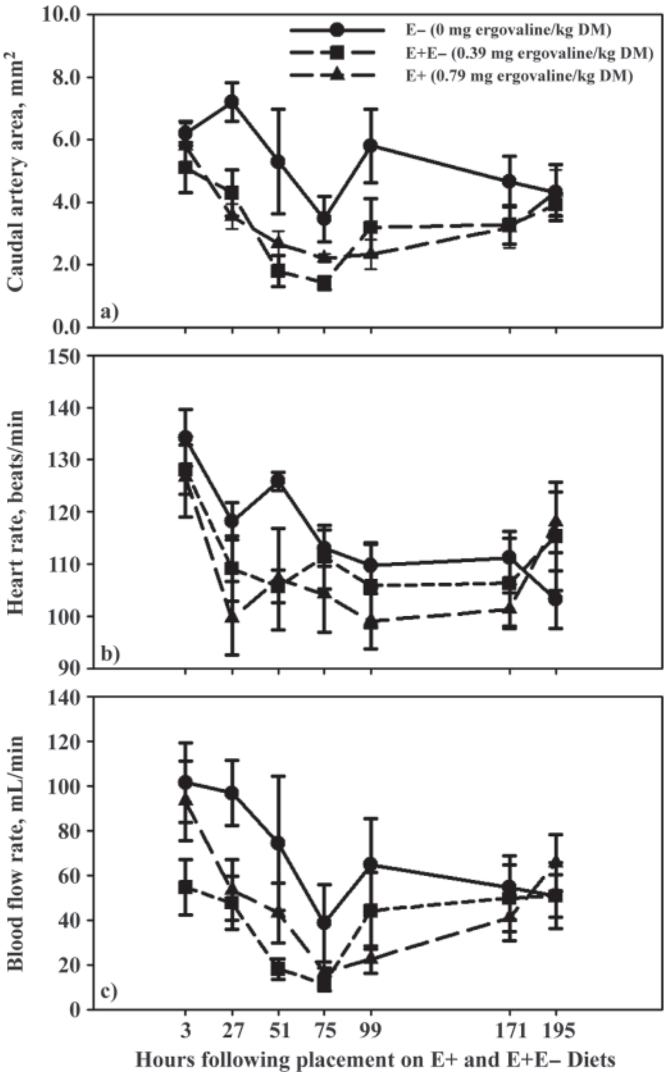

Figure 4. Caudal artery luminal areas (a), heart rates (b), and blood flow rates (c) measured during the experimental period for heifers fed diets with noninfected (E−; 0 μg of ergovaline/g of DM), a 1:1 mixture of E− and endophyte-infected (E+E−; 0.39 μg of ergovaline/g of DM), or endophyte-infected (E+; 0.79 μg of ergovaline/g of DM) tall fescue seed.

Prolactin concentrations in cattle have been shown to have a positive relationship with ambient temperature (Tucker and Wetteman, 1976; Tucker et al., 1991). This indicates that deviation from baseline concentrations for E+ and E+E− was partially due to environmental effects and not completely caused by ergot alkaloids. Lack of departure from baseline measures with the E− diet, however, strongly suggests that ergot alkaloids in the E+ and E+E− diets were the major factor in reducing prolactin concentrations during the experimental period.

Doppler Measures

Caudal artery of heifers on the E+ diet exhibited vasoconstriction earlier (27 vs. 51 h) than was detected with the E+E− diet. The reduction in caudal artery luminal area from the baseline for E+ heifers at 27 h from initial feeding of the diet was not as rapid as the 4-h response time reported by Aiken et al. (2007), who fed a similar diet concentration of ergovaline (0.85 μg of ergovaline/g of DM). Cross-sectional lumen area of the caudal artery in E+ heifers remained less than the baseline area until 195 h when the greatest ambient temperature during the experimental period was recorded. Artery lumen area in E+E− heifers remained less than the baseline until 171 h after initial feeding of the diet. This increase in cross-sectional area also corresponded with an increase of approximately 4°C in mean ambient temperature from the previous day; however, maximum vasoconstriction with the E+E− diet was measured at 75 h when the mean ambient temperature was slightly less than was recorded when the 171 h measurement was taken. It is plausible there was physiological adaptation to increase caudal artery luminal area as ambient temperatures increased in the latter days of the experimental period. Caudal artery luminal area of E− heifers was less than baseline at 75 h, which likely was in response to a 5°C drop in mean ambient temperature from the previous day.

For E+ heifers, proportionate differences in heart rates from the baseline rates were consistently negative from 27 to 171 h, but significant differences were detected only at 27, 99, and 171 h after initial feeding of the experimental diets. Aiken et al. (2007) reported reductions in heart rate from 4 to 100 h after initial feeding of an E+ diet. Ambient temperatures during that experiment were generally less during the experimental period than the adjustment period when baseline measures were recorded. As previously discussed, colder temperatures during the adjustment period of the present experiment likely caused some reduction in heart rate as a reflex to maintain blood pressure during vasoconstriction (Glick and Covell, 1968; Cowley et al., 1973; Fazan et al., 2005). Heart rate for E+E− heifers remained similar to baseline measures throughout the experimental period, which indicated constriction was not to an extent that would reduce heart rate as a reflex to maintain blood pressure.

In a review of the literature, Oliver (2005) concluded that heart rate is not directly affected by ergot alkaloids, but can be reflexively decreased in response to vasoconstriction that results in greater blood pressure. There was a vasoconstriction response in the present experiment for both experimental diets, but apparently not enough to further decrease heart rates relative to baseline rates. Aiken et al. (2007) reported that reductions in heart rates in response to feeding toxic tall fescue seed had coincided with decreases in ambient temperatures that approached 0°C. These results contradict experiments that reported increases in heart rate for dairy calves fed toxic tall fescue extracts and maintained at thermoneutrality (Carr and Jacobson, 1969; Walls and Jacobson, 1970). Ergot alkaloid toxicity could interact with ambient temperature in affecting heart rate.

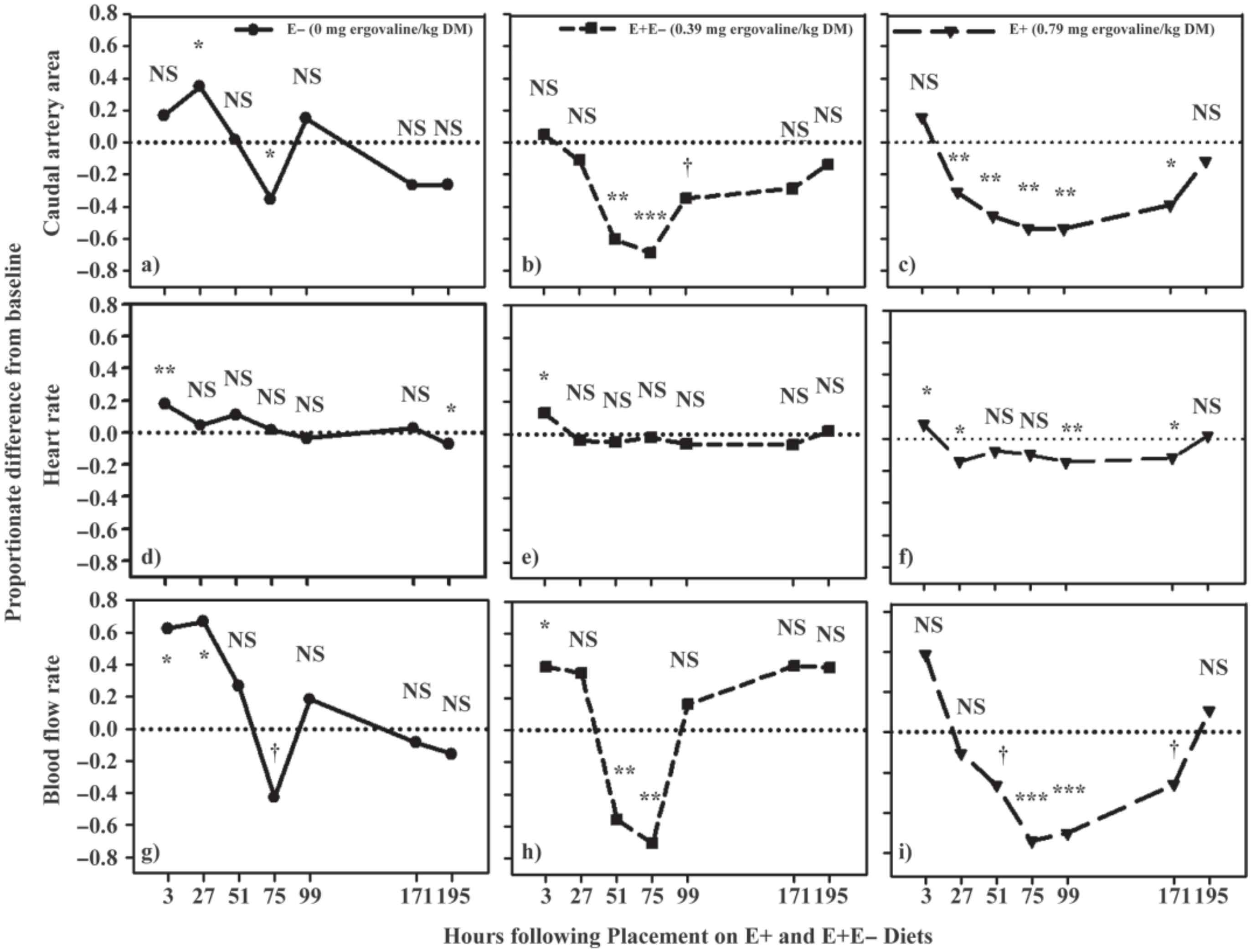

Figure 5. Proportionate differences between caudal artery luminal area (a, b, c), heart rate (d, e, f), and blood flow rate (g, h, i) responses to experimental diets and baseline measurements. Baselines (·) were measured for individual heifers during the adjustment period. Treatment diets were: a, d, g) noninfected (E−; 0 μg of ergovaline/g of DM); b, e, h) a 1:1 mixture of endophyte-infected and E− (E+E−; 0.39 μg of ergovaline/g of DM); or c, f, i) endophyte-infected (E+; 0.79 μg of ergovaline/g of DM) tall fescue seed. Statistical differences from baseline measures denoted by $*P < 0.10$; $**P < 0.05$; $***P < 0.001$; $\dagger P < 0.10$; and NS = not significant ($P > 0.10$).

Although a vasoconstriction response was detected for E+ heifers at 27 h, a reduction in blood flow rate did not occur until 51 h after the initial feeding. Although mean flow velocity at 27 h was not significantly greater than the baseline velocity, it was increased enough to compensate for the vasoconstriction that was occurring at this measurement time. This response was less rapid than the 4-h response time reported by Aiken et al. (2007). For the present experiment, blood flows at 3 h after the initial feeding of the experimental diets were above the baselines for the E− and E+E− diets. Blood flow that was measured on this day had a mean ambient temperature of 3.5°C, but was preceded by days with mean ambient temperatures below 0°C. The sudden rise in ambient temperature on the first day of the experimental period could have caused over-adjustment in caudal artery luminal area and heart rate. Further, blood flow rate in E− heifers declined below baseline only at the 75-h measurement when mean ambient temperature had declined approximately 5°C from the ambient temperature in the preceding day. The rapid declines in artery area and blood flow rate on the day that ambient temperatures substantially decreased indicated the heifers on the nontoxic diets were more responsive to ambient temperature.

Blood flow rates below the baseline for E+E− and E+ heifers beginning at 51 h indicated a similar sensitivity to the 2 ergot alkaloid concentrations. Reductions in blood flow rate from the baselines averaged 49% in the E+ heifers and 63% in the E+E− heifers; however, the vasoconstriction and blood flow rate responses to E+ persisted for 120 h, whereas they persisted for only 48 h with the E+E− diet. Although there was sensitivity of the vasculature to the reduced concentration of dietary ergot alkaloids, blood flow through the caudal artery of these heifers showed a more rapid adjustment and relaxation from the vasoconstriction state. Physiological adjustment also could have occurred with the E+ diet on the final day when vasoconstriction and blood flow rates were similar to baseline measures and

Table 1. Doppler ultrasound measures of peak systolic velocity (PSV), end diastolic pressure (EDV), mean velocity (MNV), and pulsatility index taken for the caudal artery at the fourth coccygeal vertebrae in heifers fed endophyte free (E−), endophyte-infected (E+), or a 1:1 mixture of E+E− tall fescue seed[1,2]

			Hours from initial feeding of endophyte tall fescue seed								
Item	Diet	Baseline[3]	3	27	51	75	99	171	195	SEM
PSV, cm/s	E−	19.0	21.0	20.8	20.2	19.5	18.3	20.2	19.0	0.08
	E+E−	15.3	17.6	21.1	20.5	15.0	20.1*	21.7**	19.7	0.7
	E+	19.4	22.0	23.7*	25.6	15.5**	17.4**	22.9	20.9	1.2
EDV, cm/s	E−	3.3	5.3	5.1	4.0	1.4	3.6	3.4	4.4	0.5
	E+E−	2.1	3.6	4.0	2.7	0.9**	5.7	5.6	4.2	0.6
	E+	4.2	5.8	8.0	5.7	1.3	3.6	5.3	6.1	0.5
MNV, cm/s	E−	9.4	11.8*	11.4	10.3	8.3	9.5	10.0	10.6	0.6
	E+E−	7.1	9.2*	11.2	9.9*	6.6	11.6	12.3*	10.9*	0.6
	E+	10.5	12.3	14.8	14.3	7.3*	9.3	12.6	12.6	0.8
Pulsatility index	E−	1.9	1.5	1.5**	1.7	2.4**	1.7	1.9	1.5*	0.1
	E+E−	2.3	1.7	1.8	2.0	2.4	1.6*	1.5*	1.6**	0.1
	E+	1.6	1.5	1.2**	1.4***	2.2**	1.5	1.5	1.2***	0.1

[1]Treatment diets were a) noninfected (E−; 0 μg of ergovaline/g of DM), b) a 1:1 mixture of endophyte-infected and E− (E+E−; 0.39 μg of ergovaline/g of DM), or c) endophyte-infected (E+; 0.79 μg of ergovaline/g of DM) tall fescue seed.

[2]Baselines were measured for individual heifers during the adjustment period.

[3]Baseline measures for individual pens were used for statistical analyses.

*, **, ***Percentage difference from baseline measure is significant at $P < 0.10$, $P < 0.05$, and $P < 0.01$ significance levels, respectively.

the greatest mean ambient temperature was recorded. It is plausible that ergot alkaloids bioaccumulate in the vasculature and reach a threshold before persistent vasoconstriction and lack of responsiveness to changes in ambient temperature can occur (Klotz et al., 2007).

Mean velocities with the E+ diet did not vary from the baseline, except at 75 h ($P = 0.072$) when mean velocity was significantly less than the baseline. Blood flow rate at the caudal artery was calculated as the product of artery luminal cross-sectional area, mean blood flow velocity, and heart rate; therefore, reduction in blood flow with greater ergot alkaloid concentrations was primarily associated with the vasoconstriction, except at 27, 99, and 171 h when significant declines in heart rate also affected blood flow rate. Mean velocities with the E+E− diet were generally greater during the experimental period, which indicated that reduced blood flows in these heifers at 51 and 75 h from the initial feeding were caused solely by vasoconstriction because there were no significant reductions in heart rate. These results suggest that the vasoconstrictive response to ergot alkaloids was indicated to be the major contributor in the reduction of blood flow in cattle consuming toxic tall fescue.

Ergovaline concentration of the E+ diet was slightly less than the diet concentration of 0.85 μg of ergovaline/g of DM fed to heifers by Aiken et al. (2007) to investigate blood flow responses to ergot alkaloids. Further, 0.39 μg of ergovaline/g of DM in the E+E− diet was approximately 50% of the 0.65 and 0.75 μg of ergovaline/g of DM shown to elicit toxicosis symptoms in steers (Hill et al., 1994) and sheep (Looper et al., 2007), respectively. Therefore, the threshold concentration of ergovaline to elicit toxicosis seems to be less than 0.39 μg ergovaline/g of DM.

Results indicated a vasoconstrictive response by 27 h after initiation of a diet that contained 0.79 μg of ergovaline/g of DM and by 51 h after initiation of diet that contained 0.39 μg of ergovaline/g of DM. Vasoconstriction occurred after measurement of baseline artery luminal cross-sectional areas when ambient temperatures were likely low enough to place heifers below thermoneutrality. Therefore, strength of the vascular response to ergot alkaloids allowed detection relative to an apparent response to cold ambient temperatures Although declines in blood flow rates were detected at 51 h for both experimental diets, caudal artery luminal area and flow rates adjusted up and were similar to baseline rates by 171 h for the diet with less ergovaline and by 195 h for the diet with greater ergovaline. It is concluded that vascular blood flow in cattle is sensitive to ergot alkaloids; however, longer duration trials are needed to determine if bioaccumulation of alkaloids eventually restricts an ability to adjust vascular flow rates with changes in ambient temperature.

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